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Syntheses of Cyclic Hydroxamic Acid Derivatives, and Their Chelating Abilities and Biological Activities[1)]

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Several kinds of cyclic hydroxamic acids were synthesized. They exhibited metal chelating abilities and analgesic activities, as expected. Besides these activities, some of these compounds inhibited the growth of microorganisms.

Keywords——cyclic hydroxamic acid; metal chelate; analgesic activity; antimicrobial activity; nuclear magnetic resonance; effective magnetic moment

In a previous paper,[2)] we reported the synthesis of various hydroxamic acids and their metal complexes, some of which exhibited anti-inflammatory and analgesic activities, which were suggested to be related to their metal complex-forming abilities. Among them, 3-(3,4-dimethoxyphenyl)propiohydroxamic acid (A) was more active than aspirin. These results prompted us to synthesize several kinds of heterocyclic compounds having a hydroxamic acid structure in their molecules and to test their chelating abilities and analgesic activities.

Chemistry

First of all, 1-hydroxy-2-oxo-2,3-dihydroindole (**1**)[3)] was synthesized by the reductive cyclization of 2-nitrophenylacetic acid, which was obtained from ethyl (2-nitrophenyl)-pyruvate by hydrolysis and oxidative decarboxylation successively. As expected, the cyclic hydroxamic acid (**1**) readily coordinated to copper ion, when it was mixed with an equimolar amount of copper acetate. The greenish-blue crystalline copper complex (**1**–Cu) thus obtained was composed of the ligand (**1**) and Cu(II) in a 2:1 ratio, based on the result of elemental analysis. Furthermore, the magnetic susceptibility of **1**–Cu, measured by the Gouy method[4)] at room temperature, indicated the effective magnetic moment (μ_{eff}) to be 1.84 B.M. These physical data indicated that **1**–Cu has a square-planar structure, as shown in Chart 1. The cyclic hydroxamic acid (**1**) also formed a Zn(II) complex, which was presumed from the result of elemental analysis to consist of the ligand (**1**) and Zn(II) in a 2:1 ratio; nuclear magnetic resonance (NMR) spectral data suggested the structure (**1**–Zn) as illustrated in Chart 1.

1-Hydroxy-5-oxo-4,5-dihydroimidazole derivatives (**2a**, **b**)[5)] were synthesized by the addition of hydroxylamine to azlactones,[6)] followed by recyclization with hydrochloric acid. Both **2a** and **2b** were obtained in the pure state, but their geometrical isomerism, *E* or *Z*, was not examined. When **2a** was reacted with Cu(II) acetate, Cu(II) complex precipitated as a greenish-blue powder, which was not further examined because of its instability.

2-Aminobenzohydroxamic acid was heated with acetic anhydride or with formic acid to afford 3-hydroxy-2-methyl-4-oxo-3,4-dihydroquinazoline (**3**)[7)] and 3-hydroxy-4-oxo-3,4-dihydroquinazoline (**4**),[7)] respectively. Copper(II) complex (**3**–Cu), prepared from **3** and copper acetate, was presumed to have the structure shown in Chart 2 on the basis of elemental

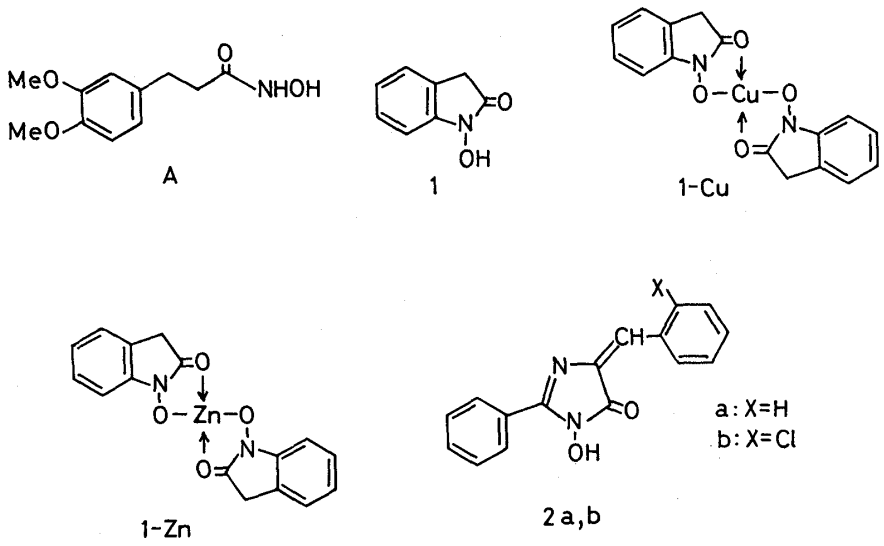


Chart 1

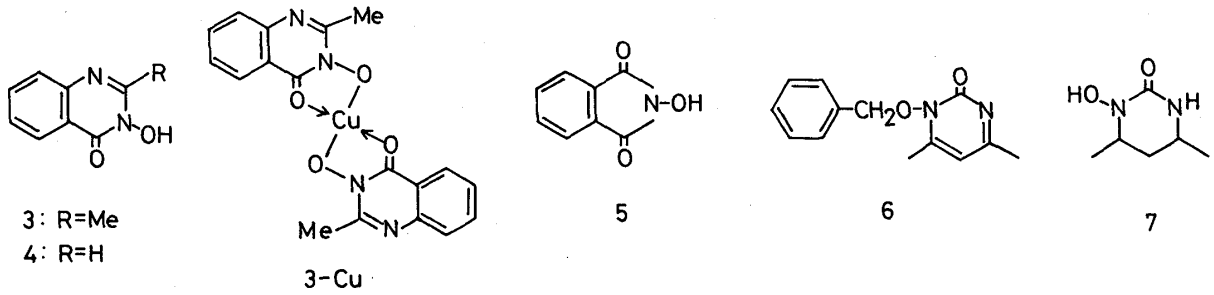


Chart 2

analysis and magnetic susceptibility measurement.

N-Benzyloxyphthalimide, prepared from phthalic anhydride and *O*-benzylhydroxylamine, was debenzylated to *N*-hydroxyphthalimide (**5**).[8] *N*-Benzyloxyurea, prepared from *O*-benzylhydroxylamine and potassium cyanate, was condensed with acetylacetone to afford 1-benzyloxy-4,6-dimethyl-2-pyrimidone (**6**).[9] In the process of catalytic reduction of **6** for debenzylation, the pyrimidine ring was unexpectedly saturated, yielding 4,6-dimethyl-1-hydroxy-3,4,5,6-tetrahydro-2-pyrimidone (**7**).

3,5,6-Trisubstituted 1-hydroxy-2-oxo-1,2-dihydropyrazines were synthesized from several kinds of amino acids and 1,2-diketones. First of all, glycinohydroxamic acid (**10a**), prepared from glycine (**8a**) *via* ethyl glycinate (**9a**), reacted readily with diacetyl at low temperature to yield 5,6-dimethyl-1-hydroxy-2-oxo-1,2-dihydropyrazine (**11a**).[10] Compound **10a** furnished 5,6-diphenyl- and 6-methyl-5-phenyl-1-hydroxy-2-oxo-1,2-dihydropyrazine (**12a** and **13a**) on heating with benzil and 1-phenyl-1,2-propanedione,[11] respectively. In the latter case, the 5-methyl-6-phenyl derivative (**13'a**), a substituent isomer of **13a**, was possibly formed. In order to clarify this problem, the condensation product (**13a** or **13'a**) was benzoylated at the hydroxy group and the NMR spectrum was examined. In the 1-benzoyloxy derivative (**14a**), the methyl proton signal was shifted to higher magnetic field, but the aromatic proton signals were unchanged. These observations suggested that the methyl group is located near the 1-hydroxy group, as shown in **13a**, and the possibility of structure **13'a** was eliminated.

By the same procedure, various 3,5,6-trisubstituted 1-hydroxy-2-oxo-1,2-dihydropyrazines were synthesized, using four other kinds of amino acids and 1,2-diketones; namely, 3-

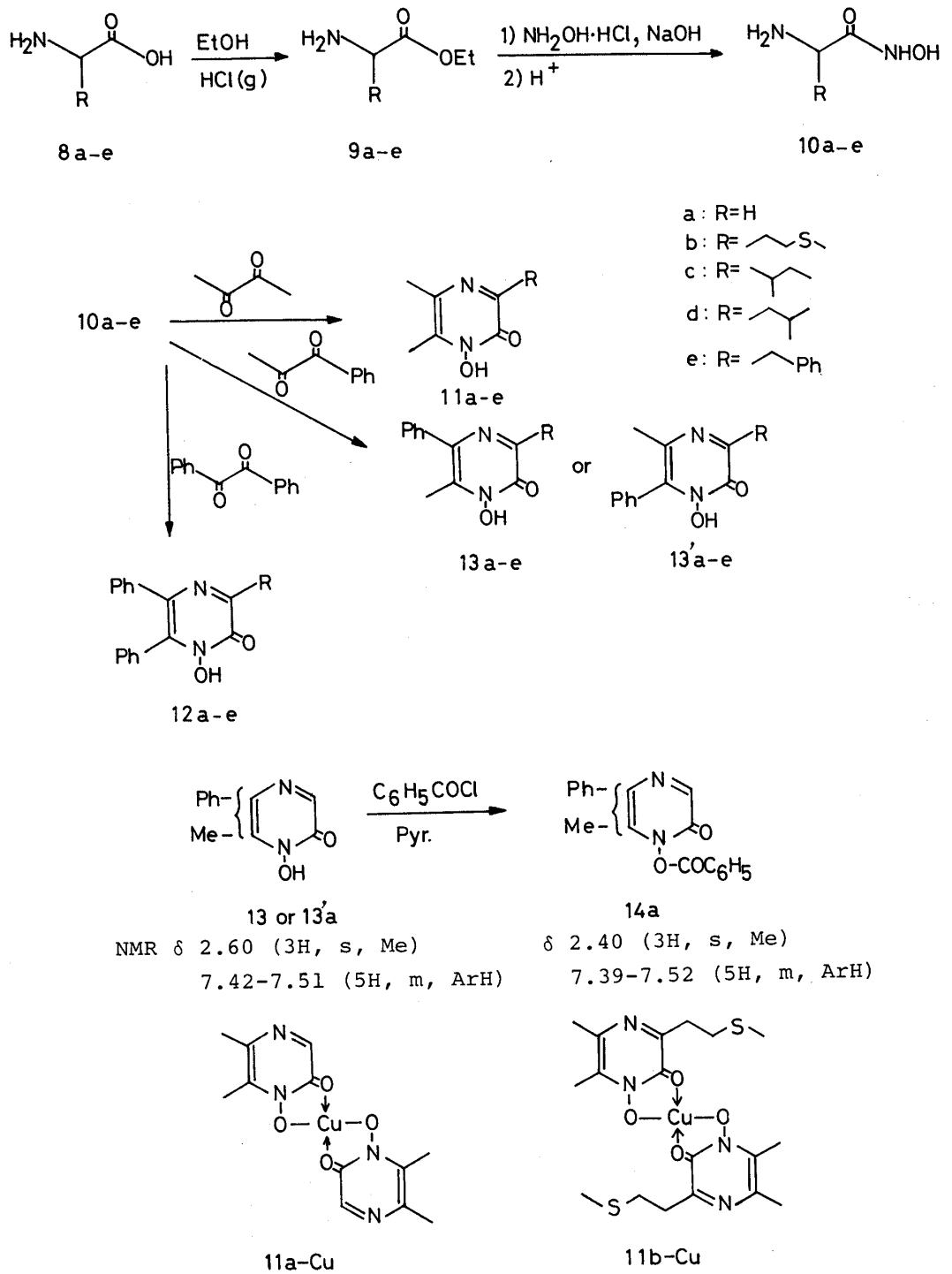


Chart 3

(3-thiabutyl) derivatives (**11b**, **12b**, **13b**) from methionine, a 3-(1-methylpropyl) derivative (**11c**) from isoleucine, a 3-(2-methylpropyl) derivative (**13d**) from leucine, and a 3-benzyl derivative (**11e**) from phenylalanine. Among them, **11a** and **11b** formed copper complexes (**11a**–Cu, **11b**–Cu), which were presumed to have the structures shown in Chart 3 from the results of elemental analyses and magnetic susceptibility measurements.

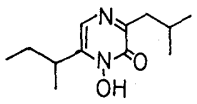
Biology

Antimicrobial Activities of 1-Hydroxy-2-oxo-1,2-dihydropyrazines——Although we synthesized cyclic hydroxamic acids in the expectation that they would have analgesic activities,

TABLE I. Antimicrobial Activities of 1-Hydroxy-2-oxopyrazines (MIC: μg/ml)

Microorganism	Compound								
	B[a]	**11a**	**13a**	**11b**	**12b**	**13b**	**11c**	**13d**	**11e**
Staphylococcus aureus FDA 209P	30[9]	25	50	12.5	>100	>100	>100	100	>100
Staphylococcus aureus 308A-1		50	100	12.5	>100	>100	>100	100	>100
Streptococcus mitis AMERICA		25	100	12.5	>100	>100	>100	>100	>100
Streptococcus faecium IFO 3128		100	100	6.25	>100	>100	>100	>100	>100
Streptococcus pneumoniae Type 1	15[9]	25	50	12.5	>100	>100	>100	>100	6.25

a) Aspergillic acid.



B

TABLE II. Analgesic Activities of Cyclic Hydroxamic Acids

Compound	Oral dose (mg/kg) (mice $n=10$)	PQ-writhing inhibition (%)
A	50	64[a]
1	50	29.5
2b	50	34.9
3	50	48.8[b]
11a	50	37.5
11b	50	46.5[b]
12b	50	33.0

a) $p<0.01$. *b*) $p<0.05$.

we were aware that aspergillic acid (B) was reported to show antimicrobial activities.[9,12] We therefore examined the antimicrobial activities of the 1-hydroxy-2-oxo-1,2-dihydropyrazines obtained above. As shown in Table I, the 5,6-dimethyl derivative (**11a**) inhibited the growth of microorganisms, but the 5-phenyl-6-methyl derivative (**13a**) was less active. The 5,6-dimethyl-3-(3-thiabutyl) derivative (**11b**) exhibited greater inhibition and a broader antimicrobial spectrum than aspergillic acid (B). The 5,6-diphenyl- and 6-methyl-5-phenyl derivatives (**12b**, **13b**) were quite inactive, whereas the 3-benzyl-5,6-dimethyl derivative (**11e**) selectively inhibited the growth of *S. pneumoniae*.

Analgesic Activities of Cyclic Hydroxamic Acids——The compounds thus synthesized were evaluated for analgesic activities by the phenylquinone writhing method in mice. As shown in Table II, some of the heterocyclic compounds tested, namely, 4-(2-chlorobenzylidene)-1-hydroxy-2-phenyl-5-imidazolone (**2b**), 3-hydroxy-2-methyl-4-oxo-3,4-dihydroquinazoline (**3**), 5,6-dimethyl-1-hydroxy-2-oxo-1,2-dihydropyrazine (**11a**), 5,6-dimethyl-1-hydroxy-2-oxo-3-(3-thiabutyl)-1,2-dihydropyrazine (**11b**) and 5,6-diphenyl-1-hydroxy-2-oxo-3-(3-thiabutyl)-1,2-dihydropyrazine (**12b**) exhibited analgesic activity as expected, but were less active than the previously synthesized 3-(3,4-dimethoxyphenyl)-propiohydroxamic acid (A). It is noteworthy that **11b**, which displayed the greatest antimicrobial activity as mentioned above, also exhibited remarkable analgesic activity. Although these cyclic hydroxamic acids exhibited both chelating abilities and analgesic activities, the potency of the latter was influenced by the structure of the heterocyclic compound carrying the hydroxamic acid moiety.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus, model MP-S3, and are

uncorrected. Infrared (IR) spectra were measured in Nujol mulls with a Hitachi 260-30 infrared spectrometer, and NMR spectra were measured with a JEOL JNM-FX200 (200 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. The magnetic susceptibility was determined at room temperature by means of a Gouy magnetic apparatus[4] with a Mettler H51AR microbalance and a Tokyo Giken MW-III electromagnet in a field of about 9000 G.

1-Hydroxy-2-oxo-2,3-dihydroindole (1)——A 6% H_2O_2 solution (137.4 ml, 0.12 mol) was added to a solution of 2-nitrophenylpyruvic acid (46.83 g, 0.224 mol) dissolved in a mixture of H_2O (312 ml) and 5 N NaOH (36.8 ml, 0.234 mol) with stirring at 28—32 °C over a period of 1.5 h. The mixture was stirred at 30 °C for 1.5 h and then acidified with 50% H_2SO_4 (203 ml, 1.06 mol). To this acidic solution, Zn dust (35.4 g) was added in small portions at 30 °C over a period of 1 h, and the reaction mixture was stirred at 50—55 °C for 2 h. The solid portion collected by filtration was dissolved in 10% Na_2CO_3 solution and insoluble ZnO was filtered off. The filtrate was acidified with concentrated HCl. The precipitates were recrystallized from EtOH, to give **1** as light brown needles (9.62 g, 28.8%). mp 198—202 °C. IR ν_{max}^{Nujol} cm^{-1}: 1685 (CO), 1620 (aromatic). NMR (DMSO-d_6) δ: 3.50 (2H, s, CH_2), 6.85—7.30 (4H, m, ArH), 10.5 (1H, s, OH). *Anal.* Calcd for $C_8H_7NO_2$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.48; H, 4.77; N, 9.40.

Cu(II) Complex of 1-Hydroxy-2-oxo-2,3-dihydroindole (1–Cu)——**1** (0.2 g, 1.3 mmol) was dissolved in EtOH (3 ml) and the solution was mixed with a solution of $Cu(OAc)_2 \cdot H_2O$ (0.24 g, 1.3 mmol) in hot water (3 ml). The mixture was heated in a steam bath at 40 °C for 1 h. After cooling, the precipitates were filtered off, then washed with H_2O and EtOH successively, yielding **1**–Cu as a dark green crystalline powder (0.156 g, 66.4%). mp >290 °C. Effective magnetic moment μ_{eff} = 1.84 B.M. *Anal.* Calcd for $C_{16}H_{12}CuN_2O_4$: C, 53.41; H, 3.36; N, 7.79. Found: C, 53.55; H, 3.46; N, 8.00.

Zn(II) Complex of 1-Hydroxy-2-oxo-2,3-dihydroindole (1–Zn)——**1** (0.226 g, 1.52 mmol) was dissolved in EtOH (3.5 ml) and the solution was mixed with a solution of $Zn(OAc)_2 \cdot 2H_2O$ (0.334 g, 1.52 mmol) in hot water (3.5 ml). The resulting colorless transparent solution was heated at 40 °C for 1 h, then allowed to stand at room temperature overnight, yielding **1**–Zn as a colorless crystalline powder, which was washed with H_2O and EtOH successively and dried *in vacuo*. Yield 0.164 g (59.6%). mp >290 °C. NMR (DMSO-d_6) δ: 3.6 (4H, s, $CH_2 \times 2$), 6.98—7.33 (8H, m, ArH). *Anal.* Calcd for $C_{16}H_{12}N_2O_4Zn \cdot 2H_2O$: C, 48.32; H, 4.05; N, 7.04. Found: C, 48.25; H, 4.08; N, 7.25.

4-Benzylidene-1-hydroxy-2-phenyl-5-imidazolone (2a)——A solution of hydroxylamine hydrochloride (1.67 g, 24 mmol) in MeOH (40 ml) was mixed with NaOMe solution (Na 1.05 g, 24 mg·atom; MeOH 40 ml) at 40 °C, and NaCl that separated during ice-cooling was filtered off. To the filtrate, azlactone (3 g, 12 mmol) was added and the reaction mixture was allowed to stand at room temperature for 3 d. Crystals that precipitated were filtered off and the filtrate was concentrated under reduced pressure to give a syrup, which was purified by SiO_2 column chromatography with $CHCl_3$: MeOH = 95 : 5. The fraction, which gave a wine color with ferric chloride solution, was separated and evaporated *in vacuo*, yielding a crystalline powder (0.992 g). This powder was refluxed with 3 N HCl (20 ml) for 10 min, to furnish crystalline **2a**, which was recrystallized from EtOH. Light yellow needles (0.314 g, 29.7%). mp 210—213 °C. IR ν_{max}^{Nujol} cm^{-1}: 1695 (CO), 1635 (aromatic). NMR ($CDCl_3$) δ: 7.25 (1H, s, =CH–), 7.5—8.48 (10H, m, ArH); 10.8 (1H, br s, OH). *Anal.* Calcd for $C_{16}H_{12}N_2O_2$: C, 72.72; H, 4.58; N, 10.60. Found: C, 72.51; H, 4.44; N, 10.85.

4-(2-Chlorobenzylidene)-1-hydroxy-2-phenyl-5-imidazolone (2b)——4-(2-Chlorobenzylidene)-2-phenyl-5-oxazolone (4.94 g, 17.4 mmol) was reacted with hydroxylamine ($NH_2OH \cdot HCl$ 4.14 g, 59.5 mmol; Na 1.37 g, 59.6 mg·atom in MeOH) by the same procedure as used in the case of **2a**. The intermediate (2.12 g) was heated with concentrated HCl (40 ml) yielding **2b** as yellow needles (0.39 g, 7.4%). mp 188—190 °C. IR ν_{max}^{Nujol} cm^{-1}: 1710 (CO), 1649 (aromatic). NMR ($CDCl_3$) δ: 7.14—8.25 (9H, m, ArH), 7.88 (1H, s, =CH–), 11.00 (1H, br s, OH). *Anal.* Calcd for $C_{16}H_{11}ClN_2O_2$: N, 9.38. Found: N, 9.27.

3-Hydroxy-2-methyl-4-oxo-3,4-dihydroquinazoline (3)——A mixture of 2-aminobenzohydroxamic acid (10.98 g, 72 mmol) and acetic anhydride (65.9 ml, 70 mmol) was heated under reflux for 20 min. After cooling, H_2O (33.7 ml) and activated carbon were added and the whole was boiled for a further 25 min, followed by filtration. The filtrate was evaporated *in vacuo* and the residue was recrystallized from EtOH. Colorless needles (7.65 g, 60.2%). mp 201—202 °C. IR ν_{max}^{Nujol} cm^{-1}: 1680 (CO), 1605 (aromatic). NMR (DMSO-d_6) δ: 2.52 (3H, s, CH_3), 7.42—8.07 (4H, m, ArH), 11.40 (1H, br s, OH). *Anal.* Calcd for $C_9H_8N_2O_2$: C, 61.36; H, 4.58; N, 15.90. Found: C, 61.29; H, 4.60; N, 15.64.

Cu(II) Complex of 3-Hydroxy-2-methyl-4-oxo-3,4-dihydroquinazoline (3–Cu)——A solution of $Cu(OAc)_2 \cdot H_2O$ (0.34 g, 1.7 mmol) in EtOH was added to an ethanolic solution of **3** (0.3 g, 1.7 mmol) and the whole was heated at 45 °C for 1 h. After cooling, the pale green crystalline powder that separated was washed with H_2O and EtOH successively. Yield 0.342 g (97%). mp >250 °C. IR ν_{max}^{Nujol} cm^{-1}: 1620 (CO), 1580 (aromatic). μ_{eff} = 1.95 B.M. *Anal.* Calcd for $C_{18}H_{14}CuN_4O_4$: C, 52.24; H, 3.41; N, 13.54. Found: C, 52.27; H, 3.41; N, 13.53.

3-Hydroxy-4-oxo-3,4-dihydroquinazoline (4)——2-Aminobenzohydroxamic acid (3 g, 19.7 mmol) was heated under reflux with formic acid (6.12 ml, 16.2 mmol) for 15 min. After the mixture had cooled, H_2O (20.4 ml) was added and the whole was boiled for 5 min and allowed to stand under ice-cooling. The product that separated was recrystallized from EtOH to afford a colorless crystalline powder (1.96 g, 61.3%). mp 200—201 °C. IR ν_{max}^{Nujol} cm^{-1}: 1660 (CO), 1600 (aromatic). NMR (DMSO-d_6) δ: 7.54—8.16 (4H, m, ArH), 8.46 (1H, s, N=CH), 11.70 (1H, br s,

OH). *Anal.* Calcd for $C_8H_6N_2O_2$: C, 59.20; H, 3.73; N, 17.28. Found: C, 59.15; H, 3.74; N, 17.17.

***N*-Hydroxyphthalimide (5)**——A mixture of phthalic anhydride (2.32 g, 15.7 mmol) and *O*-benzylhydroxylamine hydrochloride (2.5 g, 15.7 mmol) was refluxed with xylene (56.7 ml), with removal of H_2O as an azeotropic mixture until no more water could be separated. Xylene was then distilled off *in vacuo* and the residue was recrystallized from EtOH, yielding *N*-benzyloxyphthalimide as colorless needles (3.58 g, 92.3%). mp 146—148 °C. Catalytic hydrogenolysis of the *N*-benzyloxy derivative (1.98 g, 17.8 mmol) was done in the presence of Pd–C in 2-butanone (20 ml). The reaction mixture was filtered and concentrated. The residue was recrystallized from 2-butanone, yielding **5** as colorless needles (0.557 g, 43.8%). mp 228—231 °C. IR ν_{max}^{Nujol} cm^{-1}: 3150 (OH), 1790, 1710 (CO), 1610 (aromatic). NMR (DMSO-d_6) δ: 7.82 (4H, s, ArH), 10.68 (1H, s, OH). *Anal.* Calcd for $C_8H_5NO_3$: C, 58.90; H, 3.09; N, 8.59. Found: C, 59.02; H, 3.11; N, 8.61.

1-Benzyloxy-4,6-dimethyl-2-pyrimidone (6)——Potassium cyanate (2.8 g, 34.5 mmol) was added in one portion to a hot aqueous solution of *O*-benzylhydroxylamine hydrochloride (5 g, 31.3 mmol) with stirring, and the precipitates were filtered off and recrystallized from EtOH to yield *N*-benzyloxyurea as colorless needles (4.3 g, 82.6%). mp 141—143 °C. Acetylacetone (1.792 g, 17.9 mmol) and H_2SO_4 (1.96 ml) were added to a solution of *N*-benzyloxyurea (2.975 g, 17.9 mmol) in dry ether (30 ml) with stirring. The reaction product crystallized out during 1 h. It was filtered off and washed with ether. The sulfate (**6**–H_2SO_4) (3.082 g, 9.4 mmol) thus obtained was added to 5% NaOH solution (15 ml) and the deposited free base was filtered off and recrystallized from H_2O to provide **6** as colorless plates (1.926 g, 46.7%). mp 131—132 °C. IR ν_{max}^{Nujol} cm^{-1}: 1660 (CO), 1600, 1590 (aromatic). NMR ($CDCl_3$) δ: 2.09 (3H, s, CH_3), 2.32 (3H, s, CH_3), 5.30 (2H, s, CH_2), 5.90 (1H, s, =CH), 7.26—7.46 (5H, m, ArH).

4,6-Dimethyl-1-hydroxy-3,4,5,6-tetrahydro-2-pyrimidone (7)——Hydrogenation of **6** (1.77 g, 7.69 mmol) was performed in the presence of Pd–C catalyst in MeOH. The filtrate was evaporated *in vacuo* and the residue was recrystallized from tetrahydrofuran (THF) to afford **7** as colorless prisms (0.361 g, 32.6%). mp 191—193 °C. IR ν_{max}^{Nujol} cm^{-1}: 3230, 3100 (OH), 1660 (CO). NMR (DMSO-d_6) δ: 1.02 (3H, d, J=4 Hz, CH_3), 1.14 (3H, d, J=4 Hz, CH_3), 1.36 (1H, m, NHC<u>H</u>), 1.96 (2H, dd, J=9, 4 Hz, CH_2), 3.40 (1H, m, N(OH)C<u>H</u>), 6.17 (1H, br s, NH), 8.39 (1H, s, OH). *Anal.* Calcd for $C_6H_{12}N_2O_2$: N, 19.43. Found: N, 19.35.

General Synthetic Methods for 1-Hydroxy-2-oxo-1,2-dihydropyrazine Derivatives——Method A: An aminoacetohydroxamic acid (**10a—e**) was dissolved in MeOH–H_2O (1 : 1) and the mixture was cooled to −30 °C in a dry ice-acetone bath and mixed with an MeOH solution of diacetyl cooled to −30 °C. To this mixture, 5 N NaOH was added dropwise with stirring at −30 °C over a period of 5 min, and the reaction temperature was elevated to −10 °C over 1 h. The mixture was allowed to stand overnight and then acidified with concentrated HCl to pH 3, followed by the removal of MeOH by distilation *in vacuo*. The residue was extracted twice with boiling $CHCl_3$. The extract was dried over Na_2SO_4 and concentrated. The residue was recrystallized from EtOH–hexane to yield the corresponding 3-substituted 5,6-dimethyl-1-hydroxy-2-oxo-1,2-dihydropyrazine (**11a—e**).

Method B: A mixture of an aminoacetohydroxamic acid (**10a—e**) and benzil in EtOH–H_2O (1 : 1) was refluxed for 17 h and EtOH was distilled off in reduced pressure. The corresponding 3-substituted 5,6-diphenyl-1-hydroxy-2-oxo-1,2-dihydropyrazine (**12a—e**) thus obtained was recrystallized from an appropriate solvent.

Method C: A mixture of an aminoacetohydroxamic acid (**10a—e**) and 1-phenyl-1,2-propanedione in EtOH–H_2O (1 : 1) was heated under reflux for 10 h and then the EtOH was distilled off. The residue was recrystallized from an appropriate solvent to yield 3-substituted 1-hydroxy-6-methyl-2-oxo-5-phenyl-1,2-dihydropyrazine (**13a—e**).

5,6-Dimethyl-1-hydroxy-2-oxo-1,2-dihydropyrazine (11a)——**11a** was obtained from glycinohydroxamic acid (**10a**) (5.2 g, 57.7 mmol) and diacetyl (5.8 g, 67.4 mmol) by method A as light brown needles (0.8 g, 13.6%). mp 145—149 °C. IR ν_{max}^{Nujol} cm^{-1}: 1640 (CO), 1590 (aromatic). NMR ($CDCl_3$) δ: 2.41 (3H, s, CH_3), 2.50 (3H, s, CH_3), 7.59 (1H, br s, OH), 8.12 (1H, s, =N–OH). *Anal.* Calcd for $C_6H_8N_2O_2$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.31; H, 5.71; N, 19.91.

Cu(II) Complex of 5,6-Dimethyl-1-hydroxy-2-oxo-1,2-dihydropyrazine (11a–Cu)——An ethanolic solution of **11a** (0.1 g, 0.71 mmol) was mixed with an aqueous solution of $Cu(OAc)_2 \cdot H_2O$ (0.142 g, 0.71 mmol) at 40 °C and the whole was stirred for 1 h. The precipitates were filtered off and washed with hot H_2O and EtOH successively, followed by recrystallization from $CHCl_3$–EtOH. **11a**–Cu was obtained as green needles (0.072 g, 59.5%). mp >290 °C. IR ν_{max}^{Nujol} cm^{-1}: 1600 (CO), 1520 (aromatic). *Anal.* Calcd for $C_{12}H_{14}CuN_4O_4$: C, 42.17; H, 4.13; N, 16.39. Found: C, 41.92; H, 4.00; M, 16.59.

5,6-Diphenyl-1-hydroxy-2-oxo-1,2-dihydropyrazine (12a)——**12a** was obtained from glycinohydroxamic acid (**10a**) (2.17 g, 24 mmol) and benzil (4.2 g, 20 mmol) by method B as colorless needles (from dimethyl sulfoxide (DMSO)–H_2O) (2.28 g, 42.1%). mp 240—245 °C. IR ν_{max}^{Nujol} cm^{-1}: 1645 (CO), 1530 (aromatic). NMR (DMSO-d_6) δ: 7.18—7.42 (11H, m, ArH, NOH), 8.27 (1H, s, N=CHCO). *Anal.* Calcd for $C_{16}H_{12}N_2O_2$: N, 10.62. Found: N, 10.43.

1-Hydroxy-6-methyl-2-oxo-5-phenyl-1,2-dihydropyrazine (13a)——**13a** was obtained from glycinohydroxamic acid (**10a**) (0.991 g, 11 mmol) and 1-phenyl-1,2-propanedione (1.603 g, 11 mmol) by method C as yellow plates (from EtOH) (1.127 g, 50.7%). mp 185—188 °C. IR ν_{max}^{Nujol} cm^{-1}: 1625 (CO), 1580 (aromatic). NMR ($CDCl_3$) δ: 2.60 (3H, s, CH_3), 7.4—7.5 (5H, m, ArH), 7.64 (1H, br s, OH), 8.30 (1H, s, N=CHCO). *Anal.* Calcd for $C_{11}H_{10}N_2O_2$: C, 65.34; H, 4.98; N, 13.85. Found: C, 65.32; H, 4.98; N, 13.75.

1-Benzyloxy-6-methyl-2-oxo-5-phenyl-1,2-dihydropyrazine (14a)——Benzoyl chloride (0.17 g, 12 mmol) was ad-

ded dropwise to a solution of **13a** (0.2 g, 10 mmol) in pyridine with stirring and ice-cooling. The reaction mixture was stirred at room temperature for 1 h and then benzene–hexane was added to furnish **14a** as colorless plates (0.269 g, 91.5%). mp 159—161 °C. NMR ($CDCl_3$) δ: 2.4 (3H, s, CH_3), 7.37—7.52 (5H, m, C_6H_5CN), 7.62—8.27 (5H, m, C_6H_5CO), 8.38 (1H, s, N=CH).

5,6-Dimethyl-1-hydroxy-2-oxo-3-(3-thiabutyl)-1,2-dihydropyrazine (11b)——**11b** was obtained from methionohydroxamic acid (**10b**) (14.25 g, 87 mmol) and diacetyl (8.7 g, 102 mmol) by method A as light yellow needles (from 2-propanol) (4.551 g, 24.5%). mp 102—103 °C. IR ν_{max}^{Nujol} cm^{-1}: 1625 (CO), 1540 (aromatic). NMR ($CDCl_3$) δ: 2.16 (3H, s, S–CH_3), 2.39 (3H, s, CH_3C–N), 2.45 (3H, s, CH_3C–NOH), 2.92 (2H, t, J=4 Hz, $C\underline{H}_2CH_2S$–), 3.15 (2H, t, J=4 Hz, –CH_2S–), 8.00 (1H, br s, OH). *Anal.* Calcd for $C_9H_{14}N_2O_2S$: C, 50.45; H, 6.59; N, 13.07. Found: C, 50.42; H, 6.62; N, 12.87.

Cu(II) Complex of 5,6-Dimethyl-1-hydroxy-2-oxo-3-(3-thiabutyl)-1,2-dihydropyrazine (11b–Cu)——An aqueous solution of $Cu(OAc)_2$ (0.297 g, 1.63 mmol) was added to an ethanolic solution of **11b** (0.35 g, 1.63 mmol) at 40 °C, and the mixture was stirred for 1 h. The precipitates were filtered off and washed with hot H_2O and EtOH successively. Green crystalline powder (from benzene) (0.304 g, 76%). mp 184—186 °C, μ_{eff}=1.94 B.M. IR ν_{max}^{Nujol} cm^{-1}: 1580, 1520. *Anal.* Calcd for $C_{18}H_{26}CuN_4O_4S_2$: C, 44.11; H, 5.35; N, 11.43. Found: C, 44.02; H, 5.31; N, 11.55.

5,6-Diphenyl-1-hydroxy-2-oxo-3-(3-thiabutyl)-1,2-dihydropyrazine (12b)——**12b** was synthesized from methionohydroxamic acid (**10b**) (3.95 g, 24 mmol) and benzil (4.2 g, 20 mmol) by method B as colorless needles (from $CHCl_3$) (2.475 g, 30.4%). mp 209—212 °C. IR ν_{max}^{Nujol} cm^{-1}: 1625 (CO), 1595 (aromatic). NMR (DMSO-d_6) δ: 2.12 (3H, s, SCH_3), 2.94 (2H, t, J=4 Hz, $C\underline{H}_2CH_2S$–), 3.24 (2H, t, J=4 Hz, CH_2S), 7.15 (5H, m, C_6H_5C–N), 7.34 (6H, m, C_6H_5–CNOH). *Anal.* Calcd for $C_{19}H_{18}N_2O_2S$: C, 67.43; H, 5.36; N, 8.28. Found: C, 67.18; H, 5.14; N, 8.12.

1-Hydroxy-6-methyl-2-oxo-5-phenyl-3-(3-thiabutyl)-1,2-dihydropyrazine (13b)——**13b** was obtained from methionohydroxamic acid (**10b**) (2.46 g, 15 mmol) and 1-phenyl-1,2-propanedione (2.22 g, 15 mmol) by method C as yellow needles (from EtOH–H_2O) (0.8 g, 13.6%). mp 118—120 °C. IR ν_{max}^{Nujol} cm^{-1}: 1635 (CO), 1580, 1560 (aromatic). NMR ($CDCl_3$) δ: 2.16 (3H, s, SCH_3), 2.55 (3H, s, $C\underline{H}_3C$–NOH), 2.97 (2H, t, J=4 Hz, $C\underline{H}_2CH_2S$), 3.24 (2H, t, J=4 Hz, CH_2S), 7.39—7.50 (5H, m, ArH), 7.78 (1H, br s, OH). *Anal.* Calcd for $C_{14}H_{16}N_2O_2S$: C, 60.85; H, 5.84; N, 10.14. Found: C, 60.71; H, 5.79; N, 10.13.

5,6-Dimethyl-1-hydroxy-3-(1-methylpropyl)-2-oxo-1,2-dihydropyrazine (11c)——**11c** was obtained from isoleucinohydroxamic acid (**10c**) (9.588 g, 66 mmol) and diacetyl (6.6 g, 78 mmol) by method A as colorless needles (from EtOH) (0.34 g, 2.6%). mp 80—85 °C. IR ν_{max}^{Nujol} cm^{-1}: 1615, 1550. NMR ($CDCl_3$) δ: 0.85 (3H, t, J=4 Hz, $CH_2C\underline{H}_3$), 1.22 (3H, d, J=4 Hz, $CHC\underline{H}_3$), 1.58 (1H, m, –CH–), 1.83 (2H, m, $C\underline{H}_2CH_3$), 2.35 (3H, s, CH_3C–N), 2.42 (3H, s, $C\underline{H}_3C$–NOH), 7.0 (1H, br s, OH). *Anal.* Calcd for $C_{10}H_{16}N_2O_2$: N, 14.27. Found: N, 13.94.

1-Hydroxy-6-methyl-3-(2-methylpropyl)-2-oxo-5-phenyl-1,2-dihydropyrazine (13d)——**13d** was obtained from leucinohydroxamic acid (**10d**) (2.193 g, 15 mmol) and 1-phenyl-1,2-propanedione (2.22 g, 15 mmol) by method C as light yellow needles (from EtOH) (54 mg, 1.4%). mp 136—138 °C. IR ν_{max}^{Nujol} cm^{-1}: 1715 (CO), 1605, 1590 (aromatic). *Anal.* Calcd for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.72; H, 7.06; N, 10.72.

3-Benzyl-5,6-dimethyl-1-hydroxy-2-oxo-1,2-dihydropyrazine (11e)——**11e** was obtained from phenylalaninohydroxamic acid (**10e**) (15.466 g, 86 mmol) and diacetyl (8.6 g, 100 mmol) by method A as light orange needles (from EtOH) (1.78 g, 6%). mp 110—113 °C. NMR ($CDCl_3$) δ: 2.36 (3H, s, CH_3C–N), 2.42 (3H, s, $C\underline{H}_3C$–NOH), 4.15 (2H, s, CH_2Ar), 5.14 (1H, br s, OH), 7.15—7.30 (5H, m, ArH). *Anal.* Calcd for $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.96; H, 6.09; N, 11.94.

Antimicrobial Activity Test——The minimum inhibitory concentration (MIC, μg/ml) was measured as follows: bouillon agar (9 ml) was mixed with 1 ml of aqueous solution containing a test compound (dissolved in small amounts of *N,N*-dimethylformamide and acetone) at various concentrations. The agar was then poured into a Petri dish. After solidification, the agar was streaked with test organism suspension and incubated at 33 °C for 18—20 h. The MIC for each compound was defined as the lowest concentration inhibiting the growth of the test organisms.

Analgesic Assay; Phenylquinone Writhing Test——Male mice weighing 17—24 g were given as aqueous solution of 0.02% phenylquinone (dissolved by adding 5% EtOH) i.p. in a volume of 1 ml/100 g body weight, 30 min after administration of the test compound. For 20 min after this phenylquinone injection, the frequency of writhing and stretching was counted.

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References and Notes

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